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Abstract: The practice of "microdosing", or the use of repeated, very low doses of LSD to improve mood or cognition, has received considerable public attention, but empirical studies are lacking. Controlled studies are needed to investigate both the therapeutic potential and the neurobiological underpinnings of this pharmacologic treatment. Methods. The present study was designed to examine the effects of a single low dose of LSD (13 micrograms) vs placebo on resting-state functional connectivity and cerebral blood flow in healthy young adults. Twenty men and women, aged 18-35, participated in two fMRI scanning sessions in which they received placebo or LSD under double-blind conditions. During each session, the participants completed drug effect and mood questionnaires, and physiological measures were recorded. During expected peak drug effect, they underwent resting-state BOLD and ASL scans. Cerebral blood flow as well as amygdala and thalamic connectivity were analyzed. Results. LSD increased amygdala seed-based connectivity with the right angular gyrus, right middle frontal gyrus, and the cerebellum, and decreased amygdala connectivity with the left and right postcentral gyrus and the superior temporal gyrus. This low dose of LSD had weak and variable effects on mood, but its effects on positive mood were positively correlated with the increase in amygdala – middle frontal gyrus connectivity strength. Conclusions. These preliminary findings show that a very low dose of LSD, which produces negligible subjective changes, alters brain connectivity in limbic circuits. Additional studies, especially with repeated dosing, will reveal whether these neural changes are related to the drug's purported antidepressant effect. NCT03790358

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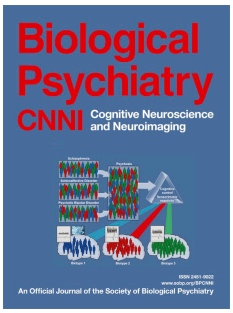


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# Preliminary report on the effects of a low dose of LSD on resting state amygdalar functional connectivity

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Abstract

*Background*. The practice of "microdosing", or the use of repeated, very low doses of LSD to improve mood or cognition, has received considerable public attention, but empirical studies are lacking. Controlled studies are needed to investigate both the therapeutic potential and the neurobiological underpinnings of this pharmacologic treatment. *Methods*. The present study was designed to examine the effects of a single low dose of LSD (13 micrograms) vs placebo on resting-state functional connectivity and cerebral blood flow in healthy young adults. Twenty men and women, aged 18-35, participated in two fMRI scanning sessions in which they received placebo or LSD under double-blind conditions. During each session, the participants completed drug effect and mood questionnaires, and physiological measures were recorded. During expected peak drug effect, they underwent resting-state BOLD and ASL scans. Cerebral blood flow as well as amygdala and thalamic connectivity were analyzed. *Results*. LSD increased amygdala seed-based connectivity with the right angular gyrus, right middle frontal gyrus, and the cerebellum, and decreased amygdala connectivity with the left and right postcentral gyrus and the superior temporal gyrus. This low dose of LSD had weak and variable effects on mood, but its effects on positive mood were positively correlated with the increase in amygdala – middle frontal gyrus connectivity strength. *Conclusions*. These preliminary findings show that a very low dose of LSD, which produces negligible subjective changes, alters brain connectivity in limbic circuits. Additional studies, especially with repeated dosing, will reveal whether these neural changes are related to the drug's purported antidepressant effect. NCT03790358

## Introduction

Depressive disorders are among the most prevalent psychiatric conditions. More than 20% of adults in the US meet criteria for a major depressive disorder during their lifetime (1). Although existing antidepressant medications, especially selective serotonin reuptake inhibitors (SSRIs), are effective in many patients, these drugs have a number of limitations, including unwanted side effects, delay of weeks to take effect and variable effectiveness across individuals. Therefore, new medications are needed to manage symptoms of depression. In the last 10 years there have been numerous anecdotal reports of the therapeutic effects of very low doses of lysergic acid diethylamide (LSD; 10-15 micrograms; µg) taken once every 3-4 days (2-5). The doses are about $1/10^{th}$ of the dose typically used to produce perceptual distortions. 'Microdosing' of LSD is widespread and usually occurs without medical supervision (2). Purported benefits include enhanced mood, improved cognition, as well as relief from migraine headaches, pre-menstrual symptoms and traumatic brain injury (2). Yet, the effects of such low doses of LSD have only recently become the subject of scientific investigation (6) and to our knowledge there have been no controlled studies of their purported therapeutic effects. Importantly, the effects of microdoses of LSD on brain function are also not known.

LSD is a serotonin receptor agonist that acts at serotonin receptors, especially 5-HT-$2_{A/C}$, and at higher doses also dopamine (7). However, the receptor actions of very low doses of LSD are less well understood (7, 8). At higher doses, LSD alters connectivity within cortico-striatal-thalamo-cortical feedback loops, pathways implicated in gating of sensory and sensorimotor information, pinpointing the thalamus and its cortical and subcortical connections as important for psychedelic-induced effects (9-12). Relatively little is known about the actions of the drug at very low doses.

One proposed mechanism underlying the potential antidepressive effects of psychedelics is alteration in amygdala reactivity, activity, and connectivity (13, 14). Using a higher dose of LSD

(100 µg vs placebo), Mueller (15) found that LSD reduced reactivity of the amygdala and the medial prefrontal cortex during the presentation of fearful faces, a mechanism that could contribute to reducing the negative bias experienced by depressed patients (16). Furthermore, Preller (11) showed increased global brain connectivity in the amygdala after a similar dose of LSD, pinpointing the amygdala as a key target of psychedelic-induced increases in serotonin 2A receptor signaling.

The present study was designed to examine the neural effects of a low dose of LSD (13 µg) on resting state cerebral blood flow (CBF) and connectivity in healthy adults, using fMRI. The 13 µg dose was selected based on a preliminary dose-ranging study assessing the subjective and cardiovascular effects of low acute LSD (17). This dose produced minimal effects, and thus might be suitable for use in a naturalistic setting and for repeated dosing. Coincidentally, microdose users in a large survey identified 13 µg as the most effective dose for improving mood and cognition (3). Based on previous studies pinpointing the importance of amygdala and thalamic connectivity for the effects of higher doses of LSD and psilocybin (13-15, 18, 19) we specifically investigated thalamus- and amygdala seed-based connectivity and its relationship to alterations in positive and negative mood. Although the participants in this study were not depressed, we reasoned that the pattern of neural activation might shed light on the processes by which the drug may have beneficial effects in symptomatic volunteers.

## Methods

### Study Design

This study used a within-subject, double blind design consisting of two sessions wherein healthy young adults received, in random order, 0 (placebo) or 13 µg of LSD. Subjective mood states and physiological measures were recorded at baseline before drug administration and at 60 min intervals after drug administration. During the time of peak drug effect (90 min after drug

administration) subjects underwent fMRI scanning. After scanning, participants completed behavioral measures complementary to those assessed in the scanner.

Subjects

Healthy subjects (N=20, 10 women) aged 18 - 35 were recruited through flyers and online advertisements. Screening consisted of a physical examination, electrocardiogram, modified Structural Clinical Interview for DSM-V and self-reported health and drug-use history. Inclusion criteria were English fluency, right handed, at least a high school education, body mass index of 18–32 and at least one prior use of a psychedelic drug (e.g., MDMA, LSD, psilocybin, DMT). Exclusion criteria were a history of psychosis, severe PTSD or Panic Disorder, past year Substance Use Disorder (except nicotine), pregnant or nursing, working night shifts, regular medication aside from birth control, adverse reaction to a psychedelic drug or unwillingness to use this type of drug again.

Subjects were required to abstain from drugs and medications for 48 hours before and 24 hours after each session, abstain from cannabis 7 days before and 24 hours after each session, and abstain from alcohol for 24 hours before and 12 hours after each session. They were permitted to consume their normal amounts of caffeine and nicotine up to 3 hours before the scan. Subjects were instructed to have a normal night's sleep and fast for 12 hours before the sessions. A granola bar was provided at arrival as a standardized breakfast. To minimize drug-specific expectancies, subjects were told they might receive a placebo, stimulant, sedative, or hallucinogen drug. All subjects provided informed consent prior to beginning the study procedures, which were approved by the University of Chicago Institutional Review Board.

**Procedure**

Orientation session

Subjects attended an orientation session to review the protocol, provide informed consent, receive pre-session instructions and practice study tasks and questionnaires.

Experimental sessions

Subjects attended two 5-hour sessions beginning at 9 am, separated by at least 7 days. Compliance to drug abstention was verified by urinalysis (CLIAwaived Instant Drug Test Cup, San Diego, CA) and breath alcohol testing (Alcosensor III, Intoximeters, St. Louis, MO). Female subjects provided urine samples for pregnancy tests, and were tested at any phase of the menstrual cycle. After compliance was confirmed, baseline measures of subjective state and cardiovascular function were obtained. LSD (13µg; tartrate solution in water; Organix, Inc.) or placebo (water) was administered sublingually 30 minutes after arrival under double-blind conditions in a volume of 0.5 ml. Participants held the solution under the tongue without swallowing for 60 seconds, under observation of the research assistant. Subjective and cardiovascular measures were taken at 60, 115, 180, and 240 minutes. Scans were conducted 90 minutes after drug administration, and lasted about 60 min. After the final timepoint at 240 minutes, subjects completed an end of session questionnaire and were discharged.

Drug

The drug was manufactured by Organix, Inc. (MA), and prepared in solution with tartaric acid by the University of Chicago Investigational Pharmacy. A dose of 13µg was selected to be below the threshold for hallucinatory effects (17), and within the range that is used in naturalistic settings (4).

Drug Effect Measures Mood states and subjective drug effects were assessed before and at regular intervals after drug administration using the Drug Effects Questionnaire (DEQ) (20, 21), the Addiction Research Center Inventory (ARCI) (22, 23), and the Positive and Negative Affect Schedule (PANAS) (24). The DEQ consists of five questions assessing subjective drug effects using 100mm Visual Analog Scales. Subjects indicate to what extent they feel a drug effect, like a drug effect, dislike a drug effect, feel high, or want more of what they received. The ARCI consists of 49 "True-False" questions measuring typical drug effects: A (amphetamine-like, stimulant effects), BG (benzedrine group, energy and intellectual efficiency), MBG (morphine-benzedrine group, euphoric effects), LSD (lysergic acid diethylamide, hallucinogen-like effects),

and PCAG (pentobarbital-chlorpromazine-alcohol group, sedative effects). The PANAS contains 20 five-point likert scale items with subscales for *positive affect*, and *negative affect.* At the end of each session, subjects also completed an end-of-session drug identification questionnaire and the 5 Dimensions of Altered States of Consciousness (5D-ASC) questionnaire (25). Blood pressure and heart rate were monitored every 30 min using portable blood pressure cuffs (Critikon Dinamap Plus; GE Healthcare Technologies, Waukesha, WI, USA). Body temperature was recorded using a tympanic thermometer (Braun Thermoscan 5 Digital Ear Thermometer, Braun, Kronberg, Germany).

Imaging

Imaging was performed using Phillips Achieva Quasar Dual 16 Ch 3T MRI scanner. Echo-planar imaging sensitive to the BOLD signal were acquired to measure neural responses during rest (repetition time, 2,000 ms; echo time, 29 ms; flip angle, 76°; 35 interleaved 3mm-thick axial slices; matrix, 88×88; 2.5×2.5×3.0 $mm^3$ voxels; acceleration factor 2; 180 volumes). Arterial spin labeling (ASL) data were acquired to assess for perfusion alterations that might qualify any fMRI changes observed. We used a pseudo-continuous sequence (repetition time, 4700 ms, voxel size, 3.4x3.4x5$mm^3$, field of view, 220x220x145$mm^3$, flip angle, 90°, echo time,13 ms , bolus time,180 0 ms, TI, 3600 ms) with 35 control-label pairs. A proton density-weighted image (repetition time, 10000 ms, echo time,13 ms, voxel size, 3.4x3.4x5$mm^3$) was acquired to calibrate CBF quantification. A structural scan was acquired using a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence for co-registration and normalization to the Montreal Neurological Institute coordinate system. Head movement was minimized through instructions to participants and foam padding around the head.

Data analysis:

Peak change-from-baseline values were calculated for each subject on each repeated subjective and physiologic measurement. Subjective effects were analyzed using repeated-measures analysis of variance (ANOVA) of peak change scores with dose condition as the within-subjects

factor. The primary analysis was the comparison of fMRI resting state connectivity after drug and placebo.

fMRI data were analyzed using the CONN functional connectivity toolbox 18.b (http://www.nitrc.org/projects/conn) (26). Preprocessing included functional realignment and unwarping, slice-timing correction, co-registration, structural segmentation and normalization into a standard stereotactic space (Montreal Neurological Institute), functional normalization, outlier detection (ART-based identification of outlier scans for scrubbing), and smoothing with a 8 mm full width at half maximum Gaussian kernel. Denoising included scrubbing with a global signal threshold of $z > 3$ and a composite subject motion threshold of >0.5 mm using ART as implemented in CONN. Subjects with more than 20% frames flagged for scrubbing were completely excluded from all analyses. All subjects passed these criteria. Further denoising steps were linear regression of the six motion parameters and their first derivatives, and the white matter and cerebrospinal fluid signals, using individual tissue masks obtained from the T1-weighted structural images. The resulting functional images were high-pass filtered (>0.008 Hz). After preprocessing, seed-to-voxel connectivity maps were computed for each participant. Previous studies have repeatedly shown that psychedelics alter activity and connectivity in the thalamus and the right amygdala in humans (10-14, 18, 27). The right amygdala and the bilateral thalamus were therefore selected as seed ROIs derived from the FSL Harvard-Oxford atlas with the whole brain as target. The between-conditions contrast LSD>Pla was computed on the 2$^{nd}$ level. Results were considered significant after cluster size correction ($P < 0.05$, FDR) based on a cluster-forming voxel threshold of $P < 0.001$, uncorrected. Given that the microdosing of LSD has anecdotally been reported to have antidepressant effects, we were specifically interested in the relationship between LSD-induced alterations in brain connectivity and changes in mood. To investigate this relationship, the 1$^{st}$ eigenvariate of significant clusters was extracted for each participant and correlated with changes in positive and negative mood (LSD-Pla change score) assessed with the PANAS questionnaire directly after scanning.

ASL data were preprocessed and CBF maps quantified using FSL's BASIL toolbox (28). Briefly, this included motion correction and co-registration, CBF maps quantified following Alsop (29), and normalization in MNI space. Quality was assessed following each step. For the between-drug analysis, CBF maps were globally normalized around 50 ml/100g/min and masked using an inclusive grey matter probability mask included with SPM12 set at 80%. A paired t-test was conducted on CBF within the right amygdala and bilateral thalamus first to assess change to the seed regions, and then on the gray-matter-only CBF maps using SPM12. We evaluated resulting statistical parametric maps for Pla > LSD and LSD > Pla at a significance threshold of $P < 0.05$ FWE corrected.

**Results**

Demographic characteristics

Most subjects were Caucasian (Table 1), in their twenties (mean age 25), with some college education (mean education 15 years) with some prior drug experience.

Drug response measures

LSD produced few subjective or physiological effects. Table 2 shows PANAS, ARCI and physiological measures before drug administration and immediately after the scan, and DEQ values and 5D-ASC values obtained after the scan. Marginal increases ($p<.10$) were detected on the DEQ ratings of "feel high" [$F(1,19)=3.30$, $p=0.09$], "want more" [$F(1,19)=3.69$, $p=0.07$], and "like drug" [$F(1,19)=3.23$, $p=0.09$], and the drug significantly increased scores on the sedation scale PCAG of the ARCI [$F(1,19)=8.20$, $p=0.01$]. On physiological measures, LSD
LSD significantly increased systolic blood pressure [$F(1,19)=5.91$, $p=0.03$]. LSD did not significantly alter ratings on the 5D-ASC. On placebo sessions, 16 subjects correctly identified the substance as placebo, and the remainder (1 each) guessed sedative, stimulant, opioid or

cannabinoid. On LSD sessions, 8 thought they received placebo, 7 thought they received a sedative, and 1 each labelled it stimulant, hallucinogen, opioid, cannabinoid or 'unsure'. Men and women did not differ on most measures, although women reported experiencing the drug effect earlier than men (60 vs 120 min).

*Imaging data*

*Thalamus seed-based connectivity*

LSD increased thalamus connectivity in two clusters in the cerebellum (peak voxel: x=+26, y=-70, z=-32, k=226 and x=-20, y=-72, z=-32, k=189, $p<0.05$, FDR corrected). No significant changes in thalamus connectivity were found in the cortex or subcortical structures. LSD-induced increases in thalamic-cerebellar connectivity were not correlated with changes in mood (all $p > 0.22$).

*Amygdala seed-based connectivity*

LSD increased amygdala seed-based connectivity in the right angular gyrus, right middle frontal gyrus and the left cerebellum ($p<0.05$, FDR corrected, **Fig. 1 and Table 3**). It decreased amygdala seed-based connectivity with the left and right postcentral gyrus and the superior temporal gyrus. To investigate the relationship between LSD-induced changes in mood and alterations in amygdala connectivity, we conducted an exploratory analysis correlating significant changes in connectivity strength with changes in PANAS scores (positive and negative mood) across participants. Fig. **1C** shows a significant correlation between LSD-induced changes in amygdala – mFG connectivity strength and differences in positive mood measured directly after scanning in the LSD and Pla conditions ($r=0.49$, $p<0.03$). This analysis was also conducted using the change in blood pressure as a covariate, but this did not change the results (Supplementary Data). No significant correlations were found for changes in negative mood or with alterations in connectivity strength in other brain areas.

*Cerebral Blood Flow*

Eighteen participants were included in the ASL analysis (two participants were excluded for incomplete data collection, and poor quality). No significant differences were found in CBF between placebo and LSD for the seed regions or anywhere else in gray matter.

**Discussion**

In this study, we tested the effects of a very low "microdose" of LSD (13 µg) on resting state connectivity in healthy human volunteers. We found that LSD increased amygdala seed-based connectivity with the right angular gyrus, right middle frontal gyrus, and the cerebellum, and decreased amygdala connectivity with the left and right postcentral gyrus and the superior temporal gyrus. Although the drug's effects on mood were small and variable, the increase in amygdala – middle frontal gyrus connectivity strength was positively correlated with positive mood after the drug. No changes in CBF were recorded. Despite the growing popularity of the practice of microdosing in the community and handful of studies investigating the subjective and behavioral effects of microdoses of LSD, to our knowledge this the first study to investigate the effects of a very low dose of the drug on resting state connectivity and CBF.

Our results are in line with previous studies investigating changes in amygdala responses under the influence of higher doses of LSD and other psychedelics, including psilocybin. The amygdala receives inputs from all manner of sensory information-focused areas and prefrontal cortex, and the latter is also a target of amygdala afferents, making the interpretation of results involving amygdala connectivity a complex process. Nonetheless, our study is consistent with prior work showing reduced amygdala system modulation. Using a much higher dose of LSD (100 µg compared to 13 µg) Mueller (15) reported dampened amygdala responses to fearful facial expressions and Kraehenmann (13) found similar results with the serotonergic drug psilocybin. Grimm (18) found that psilocybin not only modulated amygdala activation, but also decreased amygdala connectivity to the frontal pole during the viewing of happy faces, and decreased

amygdala connectivity with the striatum during the viewing of angry faces. Though Grimm (18) investigated task-based connectivity and our study investigated resting-state connectivity, our result that LSD decreases connectivity between the amygdala and the superior temporal gyrus, a region shown to be involved in the perception of emotions in facial stimuli (30), is of interest in light of these previous findings. Notably, however, the previous studies used substantially higher doses of the drugs, and it is of particular interest that we detected these changes in neural function at doses of LSD that produce minimal direct subjective or behavioral effects and do not alter CBF.

Amygdala hyperreactivity has been associated with a host of psychiatric disorders involving negative processing bias, including major depressive disorder and anxiety disorders (31, 32). Further, standard antidepressant medications, such as selective serotonin reuptake inhibitors (SSRIs) act acutely to dampen, and thereby normalize, disrupted amygdala responses, which may be a mechanism underlying their efficacy (33, 34). The extent to which microdoses of LSD or other serotonin agonists dampen amygdala activity and connectivity, after either acute or repeated dosing, and how these are related to mood symptoms remain an important direction for future research.

Interestingly, we also observed increased connectivity between the amygdala and middle frontal gyrus, right angular gyrus, and cerebellum. Beyond dampening amygdala responses to negative stimuli, it has been suggested that SSRIs may act by enhancing cortico-limbic connectivity in the brain, thereby facilitating appropriate emotion regulation (35). In line with these findings, in our study, changes in amygdala-middle frontal gyrus connectivity were significantly correlated with changes in positive mood. Furthermore, reduced connectivity between the right amygdala and the cerebellum has been reported in depressed patients (36). These alterations in connectivity have been suggested to play a critical role in the pathophysiology of depression given that dysfunctions in cerebellar-limbic circuits have been shown to disrupt emotional processing

(36, 37). In a future study it will be important to determine whether small doses of LSD normalize decreased amygdala-cerebellar connectivity in depressed patients.

In contrast to LSD-induced changes in amygdala connectivity, we did not find alterations in thalamo-cortical connectivity. This is not surprising given that altered information processing in thalamo-cortical loops has been suggested to underlie the psychedelic-induced altered state of consciousness (9). The “microdose” used in this study did not induce psychedelic symptoms or an altered state of consciousness and may therefore not influence these pathways, but rather change information processing in emotional networks.

Our study design had several limitations. First, our study included only healthy volunteers, and the effects of the drug on mood or brain function may be different in individuals with symptoms of depression or anxiety. Second, we investigated effects of only a single administration of LSD, whereas individuals who “microdose” the drug in real-world settings report using the drug repeatedly, every 3 or 4 days. Whether drug produces different effects on brain activity or mood after repeated doses remains to be determined. Another limitation is that we did not examine subjects’ responses to behavioral tasks implicated in mood-enhancing effects of drugs such as tasks assessing cognitive and emotion processing. Finally, perhaps because of the low dose, the effects of the drug were subtle. For example, we detected correlations between mood and connectivity in both the placebo and drug conditions. The specificity of the effect to the drug condition will await further study with additional subjects and repeated dosing.

In summary, here we report the results of the first study to investigate the effects of a very low, “microdose” of LSD on resting state functional connectivity in a sample of healthy human volunteers. We describe changes in amygdala connectivity in brain regions that are implicated in depression. It remains to be determined with repeated doses of this low-dose drug result in antidepressant effects. These promising findings provide a good basis for pursuing the efficacy of low doses of serotonin agonists in psychiatric treatment.

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**Disclosure**.

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Figure Legend

**Fig 1. Effect of LSD on Amygdala seed-based connectivity. (A)** Unthresholded cortical T-value map of the effect of LSD>Pla on amygdala seed-based connectivity. Red/orange areas indicate regions where participants exhibited stronger amygdala seed-based connectivity in the LSD condition, whereas blue/pink areas indicate regions where participants exhibited reduced Amygdala seed-based connectivity in the LSD condition compared to the Pla condition. Inlet shows the right Amygdala seed (green). Colorbar represents T-values for the contrast LSD>Pla. **(B)** Thresholded ($p<0.05$, FDR corrected) cortical map showing significant alterations in amygdala seed-based connectivity in the LSD>Pla (red areas) and Pla>LSD (blue areas) contrasts. **(C)** Correlation between amygdala – middle frontal gyrus functional connectivity (LSD – Pla) and changes in positive mood (LSD – Pla) across subjects (black data points). Grey background indicates the 95% confidence interval. Inlet shows the amygdala seed (green) and the middle frontal gyrus (red). N = 20.

Table 1. Demographic and drug use characteristics of the participants.

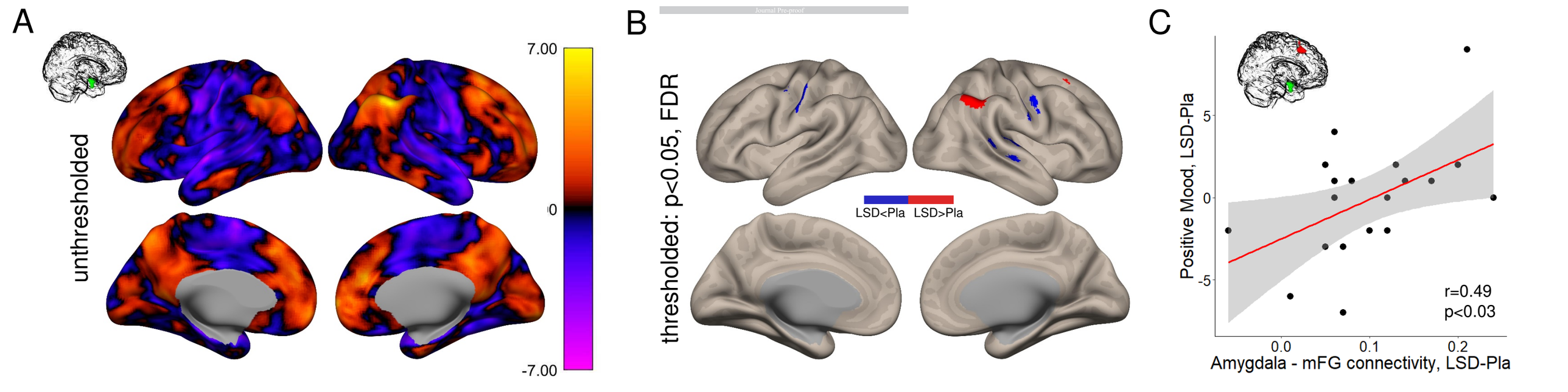
| Category | Count or Mean ± SD (Range) |
|---|---|
| **N (male/female)** | 20 (10/10) |
| **Age (years)** | 25 ± 4 (18 - 32) |
| **Education (years)** | 15 ± 1 (14 – 18) |
| **Body Mass Index (kg/m$^2$)** | 23.0 ± 3.8 (18.6 – 31.1) |
| **Race** | |
| **Caucasian** | 12 |
| **African-American** | 3 |
| **Asian** | 1 |
| **Other/ More than One Race** | 4 |
| **Depression Anxiety Stress Scale (DASS-21)** | |
| **Depression** | 2.0 ± 1.9 (0 – 6) |
| **Anxiety** | 1.4 ± 2.1 (0 – 8) |
| **Stress** | 3.3 ± 3.4 (0 – 14) |
| **Current Drug Use (past month)** | |
| **Caffeine (servings/ day)** | 1.0 ± 0.9 (0 – 2) |
| **Tobacco** | |
| **Smokers/non-smokers** | 3/17 |
| **cigarettes/ day, smokers only** | 1.3 ± 0.6 (1.0 – 2) |
| **Alcohol (drinks/ week)** | 3.6 ± 2.6 (0 – 13) |
| **Alcohol (drinking days/ week)** | 2.7 ± 1.8 (0 – 7) |
| **Cannabis (times/ month)** | 9.5 ± 9.8 (0 – 30) |
| **Lifetime Drug Use (Non-Medical; mean number of uses for users only)** | |
| **Stimulants (13 ever used)** | 16.6 ± 26.1 (1 - 100) |
| **Tranquilizer (9 ever used)** | 2.9 ± 2.3 (1 – 6) |
| **Opiate (7 ever used)** | 2.3 ± 1.9 (1 – 6) |
| **MDMA (17 ever used)** | 6.1 ± 5.8 (1 – 20) |
| **LSD (15 ever used)** | 6.7 ± 12.3 (1 – 50) |
| **Psilocybin (11 ever used)** | 4.8 ± 5.7 (1 – 20) |
| **DMT (1 ever used)** | 4 |

Table 2. Mean (and sd) values for effects of the drug. Values shown are mean scores from before and 180 minutes after drug administration (immediately after the scan). The 5D-ASC is a retrospective report completed only at the end of the session (240 min). The asterisk indicates a difference of p<.05. PANAS Positive and Negative Affect Scale; ARCI Addiction Research Center Inventory; DEQ Drug Effects Questionnaire; 5D-ASC 5 Dimensions of Altered States of Consciousness

| | Placebo Pre | Placebo Post | 13 µg LSD Pre | 13 µg LSD Post |
|---|---|---|---|---|
| PANAS | | | | |
| Positive | 25.3 (8.1) | 21.7 (9.1) | 24.7 (8.0) | 21.6 (8.6) |
| Negative | 14.5 (1.0) | 14.2 (0.4) | 14.4 (1.2) | 14.2 (0.7) |
| ARCI | | | | |
| A (amphetamine-like) | 2.4 (1.4) | 1.9 (1.1) | 2.6 (1.3) | 2.0 (1.5) |
| MBG (euphoria) | 2.5 (1.9) | 1.8 (1.5) | 2.2 (2.2) | 1.8 (1.9) |
| LSD | 2.9 (1.0) | 3.0 (1.1) | 3.3 (1.1) | 3.1 (1.1) |
| BG (stimulant-like) | 5.7 (1.1) | 5.2 (1.6) | 6.0 (1.3) | 5.4 (1.6) |
| PCAG (sedative-like) | 2.7 (1.4) | 3.9 (2.1) | 2.7 (1.2) | 5.1 (2.6)* |
| DEQ | | | | |
| Feel | | 3.6 (5.8) | | 3.5 (5.9) |
| High | | 1.4 (3.1) | | 2.0 (2.7) |
| Like | | 6.7 (14.9) | | 14.5 (21.0) |
| Dislike | | 7.7 (16.7) | | 12.1 (19.1) |
| Want more | | 6.7 (13.0) | | 16.3 (21.7) |
| 5D-ASC | | | | |
| Experienced Unity | | 0.2 (0.7) | | 0.4 (0.9) |
| Spiritual Experience | | 0.2 (0.7) | | 0.2 (0.7) |
| Blissful State | | 0.8 (2.0) | | 1.0 (3.2) |
| Insight | | 0.1 (0.4) | | 0.5 (1.4) |
| Disembodiment | | 1.5 (4.5) | | 0.4 (0.9) |
| Impaired Control and Cognition | | 0.4 (1.1) | | 0.3 (1.1) |
| Anxiety | | 0.4 (1.1) | | 0.4 (0.9) |
| Complex Imagery | | 0.4 (1.9) | | 0.3 (1.1) |
| Elementary Imagery | | 0.1 (0.2) | | 0.2 (0.8) |
| Audiovisual Synesthesia | | 0.1 (0.2) | | 0.2 (0.7) |
| Physiological Measures | | | | |
| Systolic Blood Pressure | 114.3 (9.6) | 108.9 (9.4) | 112.7 (11.3) | 114.4 (11.7)* |
| Diastolic Blood Pressure | 75.5 (12.1) | 73.9 (8.7) | 75.6 (10.9) | 76.9 (10.8) |
| Heart Rate | 70.9 (13.3) | 66.4 (11.9) | 71.0 (14.4) | 65.7 (11.5) |
| Temperature | 97.4 (0.5) | 97.7 (0.4) | 97.5 (0.3) | 97.8 (0.4) |

**Table 3.** Significant changes in amygdala seed-based connectivity.

| Brain Region | Hemisphere | x | y | z | k |
|---|---|---|---|---|---|
| *LSD>Pla* | | | | | |
| Angular Gyrus | R | 54 | -52 | 40 | 606 |
| Middle Frontal Gyrus | R | 30 | 18 | 56 | 113 |
| Cerebellum | L | -34 | -62 | -34 | 113 |
| *Pla>LSD* | | | | | |
| Postcentral Gyrus | L | -56 | -14 | 46 | 218 |
| Superior Temportal Gyrus | R | 56 | -34 | 8 | 168 |
| Postcentral Gyrus | R | 54 | -10 | 40 | 161 |



# Preliminary Report on the Effects of a Low Dose of LSD on Resting-State Amygdalar Functional Connectivity

## *Supplemental Information*

*Supplementary analysis using blood pressure as covariate*

To investigate the influence of systolic blood pressure on functional connectivity, the change in systolic blood pressure (post-scan LSD – post scan Placebo) was entered as an additional regressor in the model. The change in systolic blood pressure did not correlate with LSD-induced changes in amygdala or thalamic seed-based connectivity. Including the change in systolic blood pressure as a covariate in the thalamic-seed based connectivity analysis did not change the results. Including the change in systolic blood pressure as a covariate in the amygdala seed-based analysis revealed the results presented in Table S1. This analysis revealed a similar pattern of LSD-induced increases and decreases in amygdala connectivity as the analysis presented in Fig 1. However, three previously detected clusters did not reach significance (Middle Frontal Gyrus, Cerebellum, and Postcentral Gyrus), potentially due to reduced statistical power.

**Table S1.** Significant changes in amygdala seed-based connectivity after including changes in systolic blood pressure as covariate.

| Brain Region | Hemisphere | x | y | z | k |
|---|---|---|---|---|---|
| *LSD>Pla* | | | | | |
| Angular Gyrus | R | 56 | -52 | 40 | 549 |
| *Pla>LSD* | | | | | |
| Postcentral Gyrus | L | -60 | -14 | 40 | 158 |
| Superior Temporal Gyrus | R | 50 | -22 | 4 | 166 |